REMARKS

Claims 199-224 are added. Claims 58-98 and 100-198 have been cancelled. Support for the amendment to claims are found throughout the Specification, as filed, and no new matter is presented by the amendment.

Favorable reconsideration in light of the amendments are remarks which follow a respectfully requested.

35 U.S.C. §112 Rejections

35 U.S.C. §112, first paragraph

Claims 58-98, 100-198 have been rejected under 35 U.S.C. §112, first paragraph, on grounds that

> the specification, while being enabling for specific combinations of substances (first substance, second substance and third substance), specific liquid medium, does not reasonably provide enablement for claims as claimed with confusing terminology.

In particular, the Office asserts that:

> The specification does not in clear terms define and describe what compounds fall in each category and in examples, applicant uses a combination of phosphatidylcholine, sodium cholate and insulin (some examples contain phosphatidylglycerol in addition). According to claim 58, the solubility of the second amphipathic substance (presumably it is selected from the group consisting of surface active substances and surfactants) is greater than the solubility of the first substance". Taking applicant's examples, phosphatidylcholine appears to be the first substance and sodium cholate, the second substance; phosphatidylcholine is not soluble at all in aqueous medium and sodium cholate is highly soluble. Sodium cholate being a hydrophilic surfactant, one would expect insulin (which is also hydrophilic) to dissolve in sodium cholate and segregate in the aqueous interior and not associate with the extended surface of the second substance

The rejection is traversed.

The present application fully satisfies the requirements of 35 U.S.C. §112, including the "how to make" and "how to use" requirements of Section 112, first paragraph.

Applicants claim, in claim 199, three "classes" of amphipathic substances:
(1) at least one first surface-building amphipathic substance selected from lipids, lipid-like materials and combinations thereof capable of forming bilayers;
(2) at least one second surface-destabilising amphipathic substance selected from surface-active substances, surfactants and combinations thereof; and
(3) at least one third amphipathic substance selected from insulin, interferon, interleukin, immunoglobulin and hormone

Additionally, as set out in claim 199, the pharmaceutical composition of claim 199 is obtainable by the steps comprising selecting the at least one first and the at least one second substance, combining the first and second substances in contact with the liquid medium to form a substrate, selecting the at least one third substance, and allowing the molecules of the third substance to associate with the substrate formed by the at least one first and the at least one second substance.

By combining the first and the second substance in liquid medium **before** adding the third substance, the membrane can be formed which is suitable for association with the third substance. This membrane can work as "substrate" with which the third substance associate. This adsorption promoting capability of the substrate surface permits the adsorbing macromolecule (the third substance): i) to get enriched near the adsorbent surface, due to the locally attractive charge-charge and other interactions; ii) to optimise non-electrostatic interactions/binding to the adsorbent surface(which typically requires the presence of hydrophobic and H-bond binding sites, which are generated or made accessible by surface-flexibility and/or adaptability). See page 6, lines 25-32 of the Specification. The association of the third substance is more effective than those incorporated into the vesicle for delivering the

active agents. This feature is achieved by combining the three substances in the above described order.

As disclosed in examples 1-27 of the application, phosphatidylcholine and sodium cholate were first suspended in phosphate buffer, resulting in vesicle suspension. The suspension then handled to finally obtain highly deformable vesicles. To this vesicle suspension, protein solution containing insulin and *m*-cresol are added, resulting in carrier-insulin mixture. Results of examples 1-27 show that below insulin/lipid ratio of 6 mg/100mg total lipid (TL), 80-90 % of protein added associates with (binds to) the vesicles. Insulin binds on the surface of the vesicle formed of both the first substance and the second substance.

According to Applicants, the first substance can be selected from lipids, lipid-like materials and combinations thereof capable of forming bilayers. Disclosed examples include glycerophospholipids, namely phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositols, phosphatidic acids, and phosphatidylserines. All of these substances have extended apolar substituents in position 1 and 2, and at the phosphoryl group in position 3. Also mentioned are sphingolipids like sphingophospholipids and sphingoglycolipids. These substances also have extended apolar groups. These types of phospholipids are known to be poorly water-soluble due to their apolar portions, and are known to be capable of building membrane-like structures by aggregating upon solution or dispersion in a polar liquid medium.

Examples of materials which may be selected as the second substances are clearly defined in page 10, lines 4-20 and more specifically from page 18 line 13 to page 19 line 8. Among them, sodium cholate and Tween 80 were used in Examples. However, further specification of these substance would require inserting all of the specific substances, which is unnecessary and would unduly restrict the present invention.

Examples of materials which may be selected as the third substance include insulin, interferon, interleukin, immunoglobulin and hormone. Especially, insulin, interferon, Il-2, IgG and calcitonin were exemplified in examples.

Accordingly, applicants respectfully submit that the specification does, in fact, define and describe in clear terms what compounds fall into each category. The characteristic properties of each of these substance groups are well known in the art and therefore allow an unambiguous classification of these substances.

35 U.S.C. §112, second paragraph

Claims 58-98, 100-198 have been rejected under 35 U.S.C. §112, second paragraph, as being indefinite. In particular, the Office asserts that:

> The amended claims have the same issues, which existed before. As already indicated, if a substance is soluble in a liquid (regardless of the degree of solubility) how can it form an extended surface? What is meant by an extended surface? According to claim 58, (in other independent claims also), the molecules of the third substance associate with the extended surfaces formed by the first substance and the second substance. As pointed above, according to the examples, sodium cholate is the second substance and insulin is the third substance. How can insulin associate with sodium cholate, which is hydrophilic and would be expected to be in the interior of a liposome? Applicant's response is not adequate in this regard.

Applicants respectfully traverse and submit that the above-discussion addresses some of these issues raised by the Office.

To clarify the meaning of the term "extended surface", applicants changed the term to the "substrate", which is understood by skilled artisan from the description of the specification. The third substances bind on, i.e., associate with, the substrate.

As stated above, by combining the first and the second substance in liquid medium **before** adding the third substance, the membrane is formed which is suitable for association with the third substance. This membrane may function as a "substrate" with which the third substance

associates. This adsorption promoting capability of the substrate permits the third substance: i) to get enriched near the substrate surface, due to the locally attractive charge-charge and other interactions; ii) to optimise non-electrostatic interactions/binding to the adsorbent surface(which typically requires the presence of hydrophobic and H-bond binding sites, which are generated or made accessible by surface-flexibility and/or adaptability). See page 6, lines 25-32 of the Specification. This feature is achieved by combining the three substances in the order described above.

Upon combining the first and the second substances, the surface active molecules (second substance) are inserted into the lipid surfaces such that the surfaces are destabilized leading to an increase of the extension of the surfaces and an increase of the association capability of the substrate. However, the amount of surface-active substance must not be too high, such that only destabilization is achieved, but not complete dissolution of the surface.

More specifically, for example, single chain phospholipids, and other surfactant-like lipids, always insert their hydrophobic chains into, rather than onto, an extended surface, which forms spontaneously upon combining, for example, double-chain phospholipids with water or an aqueous solvent. Single chain phospholipids, thus, insert themselves into, but do not adsorb onto, an extended surface of amphiphilic aggregates. This is discussed in the publication by Hoyrup et al. of record The bilayer solubilising effect of such insertion is discussed in the paper by Senisterra et al.

The fact that bilayer solubilising amphipaths do not form extended surfaces in a polar solvent is generally accepted by those skilled in the art. This also includes the formation of multilayers as suggested by the Examiner. Such surfaces, which normally correspond to vesicular or lamellar phases for the surfactant like molecules, are only observed in the low water concentration region of phase diagrams, as can be seen from several ternary phase diagrams reported in Almgren.

The mechanism described by Applicants, thus, relies on surfactant insertion, for example, into lipid bilayers, and subsequent chain molecule adsorption to the extended surface of an aggregate comprising at least one surfactant-like amphipath and another surface building amphipath. The article of Almgren describes the mechanism of lipid bilayer solubilization by a number of surfactants and confirms that surfactants do not form superficial layers on top of a lipid bilayer.

35 U.S.C. §101 Rejections

Claims 186-198 have been rejected under 35 U.S.C. §101 as being a claimed recitation of use without setting forth any steps involved in the process. To expedite prosecution, the claims 186-198 are cancelled without prejudice.

35 U.S.C. §102 Rejections

WO 92/03122

Claims 58-98 and 100-198 have been rejected under 35 U.S.C. §102(b) as being anticipated by WO 92/03122. The Office asserts that WO 92/03122

> discloses a composition containing two or more amphiphilic substances with different solubilities for the administration of various active substances including insulin. Applicant indicates in the response that an English translation of the reference; the examiner however, is unable to find the translation in the electronic file. Therefore, the examiner is unable to address the specific issues argued by applicant. However, the examiner had already pointed out that the reference appears to teach the same combination of amphipathic substances together with the active agents such as insulin (see Examples 163-165-175 in the reference). A careful review of the examples in instant specification and those in the reference indicates the same components in similar amounts and the method of preparation appears to be the same.

The Office indicates that it is unable to find the translation in the electronic file, however, Applicants respectfully submit that the English translation was provided with Applicants response filed on November 19, 2003.

The rejection is traversed.

As discussed above, Applicants teach a pharmaceutical composition comprising: at least one first surface-building amphipathic substance selected from lipids, lipid-like materials and combinations thereof capable of forming bilayers; at least one second surface-destabilising amphipathic substance selected from surface-active substances, surfactants and combinations thereof; and at least one third amphipathic substance selected from insulin, interferon, interleukin, immunoglobulin and hormone. The composition is obtainable by steps comprising: selecting the at least one first and the at least one second substance, combining the first and second substances in contact with the liquid medium to form substrates, selecting the at least one third substance, and allowing the molecules of the third substance to associate with the substrate formed by the at least one first and the at least one second substance.

The pharmaceutical compositions claimed herein are processed differently than WO92/03122. This provides distinct compositions because the composition will be loaded with active agents, on the surface of the substrate, which enables more effective use of the active agent.

In particular and as discussed above, by combining the first and the second substance in liquid medium **before** adding the third substance, the membrane can be formed which is suitable for association with the third substance. This membrane can work as a "substrate" with which the third substance associate. This adsorption promoting capability of the adsorbent surface permits the adsorbing macromolecule (the third substance): i) to get enriched near the adsorbent surface, due to the locally attractive charge-charge and other interactions; ii) to optimise non-electrostatic interactions/binding to the adsorbent surface(which typically requires the presence of hydrophobic and H-bond binding sites, which are generated or made accessible by surface-flexibility and/or adaptability). See page 6, lines 25-32 of the Specification. This feature is achieved by combining the three substances in the order above.

As shown in the translation provided, WO 92/03122 does not describe or otherwise suggest the association of the third substances to adsorbent surface by combining the first and the second substance in liquid medium **before** adding the third substance, as taught by the present invention. Rather, WO 92/03122 exclusively refers to the incorporation of active agents into the described "transfersomes" and their subsequent transport through natural barriers like the skin. WO 92/03122 does not describe or otherwise suggest the association, i.e. adsorption, of the third substances specifically at the substrate as disclosed in the present application. Rather, according to the WO 92/03122 reference, droplets are formed from a solution containing the active agent. In examples of WO92/01322, active agents and the lipid suspension are combined even **before** adding a liquid buffer for lipid and surfactant in which lipid and surfactant form the vesicle (i.e., adsorbent surface). This can lead to encapsulation of the active agent in the droplet surrounded by the membrane. The agent solution then just forms the "filling" of the vesicle. Since the agent is dissolved, it may not associate with the membrane in any relevant amount.

As provided in MPEP-2131, a claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegal Bros. v. Union Oil Co. of California*, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987).

It is clear that the WO 92/03122 reference does not teach each and every element of the present claims, either expressly or inherently. In particular, WO 92/03122 does not teach a combination of substances (first substance, second substance and third substance) in a liquid medium by steps that can optimize and control the association of substances to substrate, wherein molecules of the third substance associate with the substrate formed by the first substance and the second substance.

Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Claims 58-61, 63, 66-76, 91-96, 98, 101, 117, 126-127, 157-171, 174, 186, 187 and 189-192 have been rejected under 35 U.S.C. §102(b) as being anticipated by Uster. The Office asserts that:

> Uster discloses a combination of three amphipathic substances and the formation of vesicles. This combination includes two phospholipids, PC, PG and a macromolecule, EGF. The compositions further contain cholesterol.

The office further asserts that:

> These [applicants'] arguments are not found to be persuasive since instant claim language does not exclude the neutral amino acids and others in Uster.

Applicants respectfully traverse. As set forth above, Applicants claim the combination produced by steps by which the effective association of the third substance is achieved. Especially, the third substance is selected from insulin, interferon interleukin, immunoglobulin and hormone. EGF of the Uster reference does not qualify as a third substance. Further, the third substance is associated with, not incorporated into, the substrate formed by the lipid and the surfactants.

Uster does not require the specific steps of combining the first, second and the third substances to achieve the association of the third substance on the substrate formed by the first and second substances in the presence of the liquid medium. Applicants respectfully submit that the differences between Uster and the instant compositions are now clearly reflected in the amended claims.

Accordingly, the present invention is not anticipated by Uster. Reconsideration and withdrawal of the rejection is respectfully requested.

Klibanov BBA, 1991

Claims 58-60, 63, 66-74, 91-98, 100-101, 110-122, 151-152, 171-180, 186, 189-192 have been rejected under 35 U.S.C. §102(b) as being anticipated by Klibanov. The Office asserts that

> Klibanov discloses liposomes contianing three amphipathic substances. They include phospholipids and cholesterol. The third amphipathic substance which is on the surface is either PEG or an antibody.

Applicants respectfully traverse.

The Klibanov document relates to the activity of amphipathic polyethyleneglycol in prolonging the circulation time of liposomes in the blood. The reported liposomes may consist of phosphatidylcholine and cholesterol with PEG-PE or the gangliosid GM1. In other words, the three amphipathic substances, PEG-PE and GM1, allegedly correspond to the third substance of the present invention.

However, PEG and GM1 do not fall under the third substance which is selected from insulin, interferon, interleukin, immunoglobulin and hormone. Further, throughout the entire Klibanov document it is specified that PEG-PE or GM1 are "incorporated into the membrane". For example, "incorporation of PEG-PE into the liposome membrane" is explicitly mentioned (e.g. p.144, 2nd paragraph) and incorporation of monoclonal antibody into the membrane is explicitly described (e.g. p. 145, last paragraph).

Further, Klibanov relates to the prolongation of the circulation time of liposomes and how far the substances employed for this prolongation are capable of influencing the target binding and retention of the liposomes. However, the specific steps of combining the first and second substances to form the substrate before association of the third substance are not mentioned.

Therefore, even if an antibody could fall under the class of the third substance of the present invention, Klibanov only discloses the incorporation of such substances into the membrane, which is not the subject matter of the present invention.

As set forth above, applicants claim the pharmaceutical composition obtainable by the steps of selecting the at least one first and the at least one second substance, combining the first

and second substances in contact with the liquid medium to form the substrates, selecting the at least one third substance, and allowing the molecules of the third substance to associate with the substrates formed by the at least one first and the at least one second substance.

Thus, as state above, the present invention teaches association of the specific third substances **onto** the substrate, not into the membrane and which association is achieved by the specific steps. The difference between Klibanov and instant compositions are clearly reflected in the claims.

In view thereof, reconsideration and withdrawal of the rejection are requested.

35 U.S.C. §103 Rejections

Claims 58-98 and 100-198 have been rejected under 35 U.S.C. §103(a) as being unpatentable over WO 92/03122 in further combination with either Uster (4,944,948) or Klibanov. The Office asserts that:

> The teachings of WO have been discussed above. In essence, WO basically teaches a combination of the three amphiphilic substaces claimed. What is unclear from WO is whether the third substance (macromolecule) is on the surface of the vesicles. Assuming that it is not, attaching a macromolecule on the surface, if that is desired, is deemed obvious to one of ordinary skill in the art since the reference of Uster teaches that when a macromolecule such as EGF can either be encapsulated or attached to the surface of the vesicles with the same release rates (abstract and col. 9, line 29 et seq., col. 10, line 61 et seq.) One of ordinary skill in the art would expect at least similar results. Uster further teaches on col. 4, lines 26-32 that the negative charge on the vesicles enables the EGF molecules to adsorb on the surface of the vesicle. Although Uster does not teach macro molecules other than EGF, WO teaches a variety of macro molecules and it would have been obvious to one of ordinary skill in the art based on Uster's statement that any macromolecule could be attached to the surface of the vesicles, if such is desired and with the expectation of obtaining at least similar results. One of ordinary skill in the art would be motivated to use an amphipathic molecule such as PEG since Klibanov teaches that such a use would increase the circulation time of the vesicles.

Applicants respectfully traverse.

The WO 92/03122 document reports preparations in the form of minute droplets with a membrane-like coating consisting of one or several layers of amphiphilic substances incorporating agents for the transport through barriers by maximum deformability of said droplets.

As set forth above, the WO 92/03122 document does not describet a pharmaceutical composition (first substance, second substance and third substance specifically defined in claim 199) in a liquid medium for the association of substances to the substrate, wherein molecules of the third substance associate with the substrate formed by the first substance and the second substance in a liquid medium which formation occurred prior to allowing the third substance to associate with the substrate. Further, there is no description in the WO 92/03122 reference regarding improved and controlled association of the defined third substances or the addition of surface destabilising substances for these purposes before combining the first substance and the third substance. Further, as set forth above, neither Uster nor Klibanov remedy these deficiencies.

As discussed, Uster provides sustained release formulations of EGF for the treatment of wounds. The corresponding solution is a high viscosity liposome composition, in which the specific kind of binding of the macromolecule, i.e. whether adsorbed to or entrapped in the liposome, plays no role (see col. 2, l. 30 to 31). First of all, EGF does not fall under the third substance of the present application. Further, Uster is only directed to the liposomal composition providing the sustained release. Binding of the defined third substances by taking specific steps is not addressed at all by Uster.

Applicants respectfully submit that by combining WO 92/03122 (relating to the provision of maximum deformability to liposomal formulations for the transport of agents through barriers) with Uster (being directed to high viscosity liposome compositions, which are to remain on the skin as long as possible), a person skilled in the art only learns that according to WO 92/03122, a formulation providing fast release of an agent through skin may contain surface-active substances, whereas according to Uster, formulations, which are to remain as long as possible on

the skin in order to provide sustained release may contain phospholipids mixtures as well as cholesterol and empty liposomes or EGF incorporated into the liposomes.

The same applies with respect to Klibanov, which relates to retention times in the blood circulation depending on the incorporation of different substances like PEG-PE or GM1 into a lipid membrane. Klibanov particularly relates to the prolonged retention time due to said substances and related thereto to the increase of target binding and retention of the liposomes bearing these target specific antibodies incorporated into the liposome membrane.

However, the pharmaceutical composition, as well as association, achieved by the specific steps of the present invention are not mentioned. Klibanov only describes how to incorporate substances like PEG-PE or GM1 into a lipid membrane. The present invention, on the other hand, teaches association of specific third substances onto the substrate by taking specific steps.

In view thereof, reconsideration and withdrawal of the rejection are requested.

It is believed the application is in condition for immediate allowance, which action is earnestly solicited.

Respectfully submitted,

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